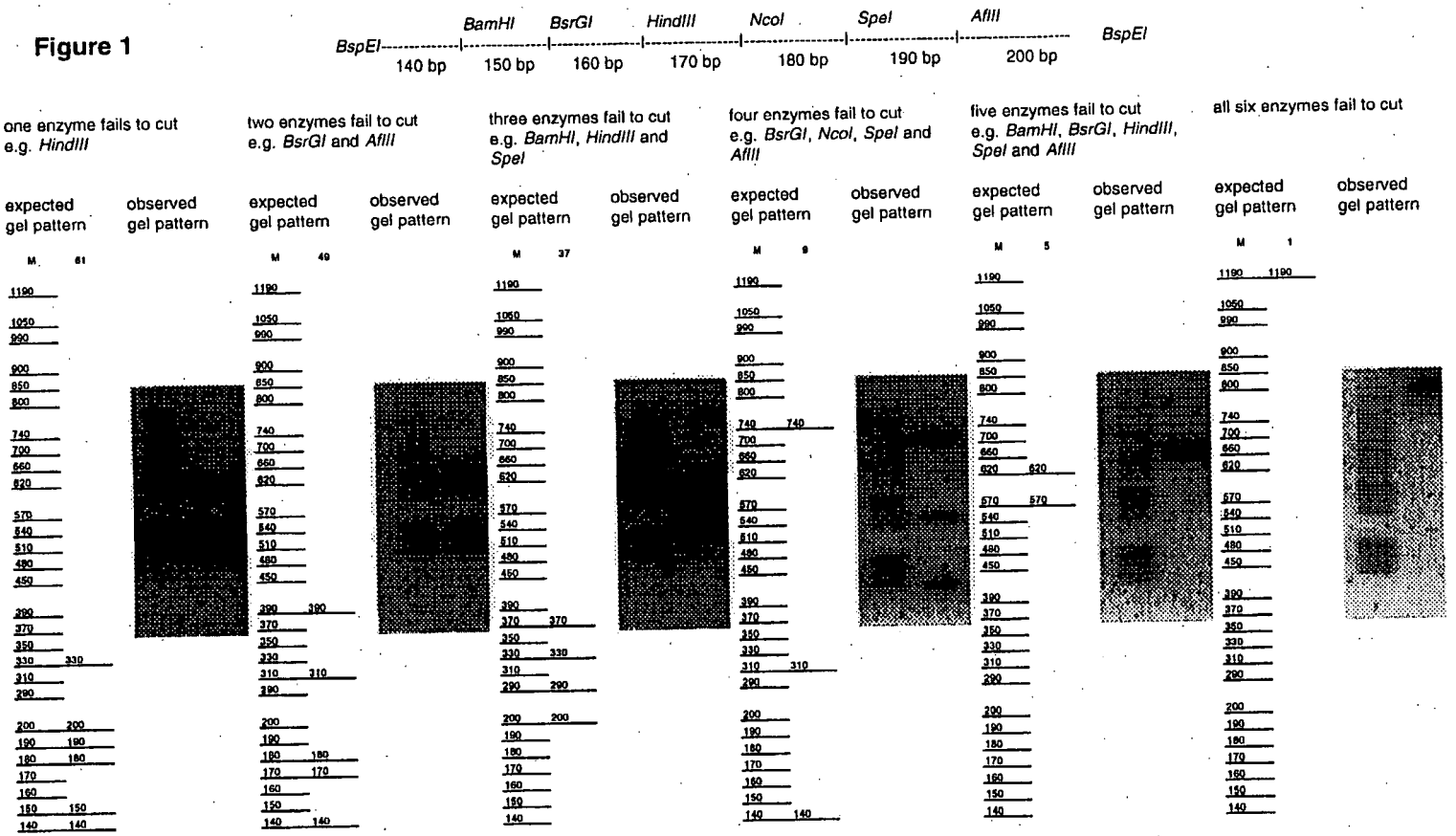
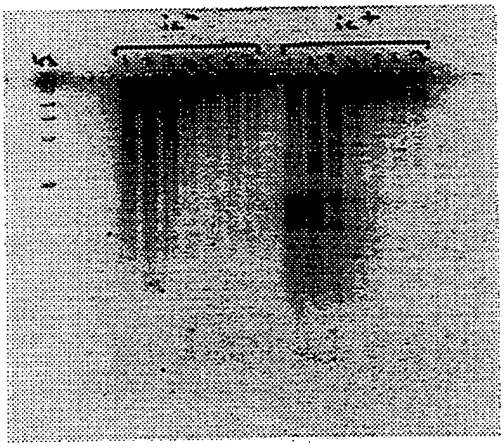
Figure 1



Figur 2

Vistra Green fluorescence

1 µg of Stratagene Kb DNA Ladder (band sizes: 250 bp, 500 bp, 750 bp, 1 kb, 1.5 kb, 2 kb, 3 kb, 4 kb, 5 kb, 6 kb, 7 kb, 8 kb, 9 kb, 10 kb and 12 kb), 3x gaps, 2 µg of canine genomic DNA digested with 0.25, 0.025, 0.0025, 0.00025, 0.000025, 0.0000025 and 0 U/µl *BamH1 / BsrGI / HindIII / NcoI / SpeI / AflII*, gap, 0.4 µg of canine genomic DNA and 1 µl of *BspEI*-released internal control DNA digested with 0.25, 0.025, 0.0025, 0.00025, 0.000025, 0.0000025 and 0 U/µl *BamH1 / BsrGI / HindIII / NcoI / SpeI / AflII*.

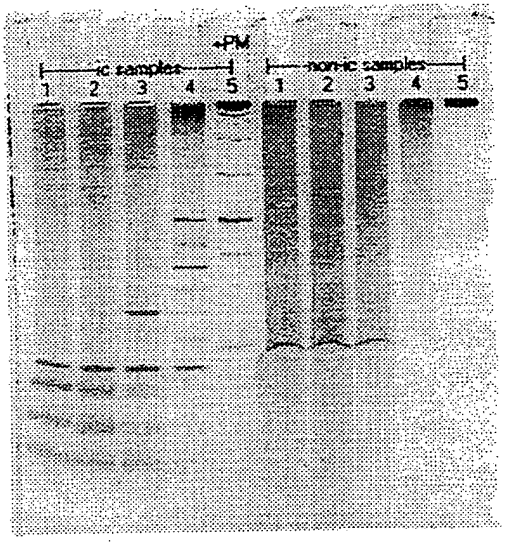


From the results above, the fragment pattern from the internal control DNA is diagnostic of both the degree of digestion and the nature of any partial restriction at less than limit digestion.

Very little digestion of canine genomic DNA and *BspEI*-released internal control DNA is observed for 0.00025 U/µl *BamH1 / BsrGI / HindIII / NcoI / SpeI / AflII* and fewer units. With 0.0025 U/µl, *BamHI* and *AflII* are both failing to cut more than the other enzymes (the 140 bp and 200 bp bands in 3ic are diagnostic of this). With 0.025 U/µl, most enzymes have cut – though not quite to completion. 0.25 U/µl thus appears to be necessary in order to achieve limit digestion of 20 µg of canine genomic DNA in a volume of 200 µl.

Figure 3

Figure 3 shows the products of the restriction digests after electrophoresis on an 8 % polyacrylamide gel. The numbers 1–5 indicate the dilution of enzyme mix used. 1=0.5 U/µl, 2=0.1 U/µl, 3=0.02 U/µl, 4=0.004 U/µl, and 5=0 U/µl. 'ic' indicates the samples that were spiked with 4 bp cutter internal control DNA. PM indicates that 180 ng of PCR molecular weight markers were added as a size standard.



All five of the partial digestion products are visible in lane ic4, between the top 130 bp band and the bottom 40 bp band, indicating that all three enzymes have failed to cut to completion at 0.004 U/µl. In lane ic3, the four complete digestion products are all visible but the 65 bp band is also present. *HaeIII* and *MboI* have digested to completion but the *MseI* digestion is still partial at 0.2 U/µl. At 0.1 U/µl and at 0.5 U/µl all three enzymes have produced limit digests.

Using the internal control spike, it is a great deal easier to determine the point at which limit digestion has occurred. The undigested internal control DNA is 130 bp. Partial digestion products generate bands of 105 bp, 90 bp, 75 bp, 65 bp, and 55 bp. The products of complete digestion generate bands of 40 bp, 35 bp, 30 bp, and 25 bp. The fragment pattern from the internal control DNA is therefore diagnostic of both the degree of digestion and the nature of any partial restriction at less than limit digestion.

Figure 4

Expected TRSPA-2 pattern for the pNW33 *BamHI*, *HindIII*, and *AflII* matrix probed with the 140 bp *BspEI* to *BamHI* fragment from pNW33

The TRSPA-2 pattern expected for matrix #7 (*BamHI*, *HindIII*, and *AflII*) hybridized with a ^{33}P-labelled probe derived from the 140 bp *BspEI – BamHI* fragment of pNW33 is shown below:

#7	*BamHI*	*HindIII*	*AflII*
BamHI	-	HYB	-
HindIII	HYB	-	-
AflII	-	-	-

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Observed TRSPA-2 pattern for the pNW33 *BamHI*, *HindIII*, and *AflII* matrix probed with the 140 bp *BspEI* to *BamHI* fragment from pNW33

The TRSPA-2 pattern observed for matrix #7 (*BamHI*, *HindIII*, and *AflII*) hybridized with a ^{33}P-labelled probe derived from the 140 bp *BspEI – BamHI* fragment of pNW33 is shown below:

#7	*BamHI*	*HindIII*	*AflII*
BamHI			
HindIII			
AflII			

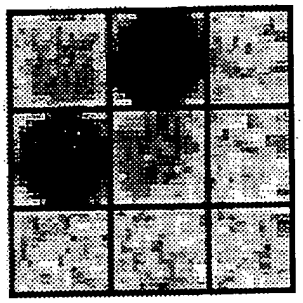

Figure 5

Expected TRSPA-2 pattern for th pNW33 *HindIII*, *NcoI*, and *SpeI* matrix probed with the 140 bp *BspEI* to *BamHI* fragment from pNW33

The TRSPA-2 pattern expected for matrix #17 (*HindIII*, *NcoI*, and *SpeI*) hybridized with a ^{33}P-labelled probe derived from the 140 bp *BspEI – BamHI* fragment of pNW33 is shown below:

#17	*HindIII*	*NcoI*	*SpeI*
HindIII	HYB	HYB	HYB
NcoI	HYB	-	-
SpeI	HYB	-	-

Observed TRSPA-2 pattern for the pNW33 *HindIII*, *NcoI*, and *SpeI* matrix probed with the 140 bp *BspEI* to *BamHI* fragment from pNW33

The TRSPA-2 pattern expected for matrix #17 (*HindIII*, *NcoI*, and *SpeI*) hybridized with a ^{33}P-labelled probe derived from the 140 bp *BspEI – BamHI* fragment of pNW33 is shown below:

#17	*HindIII*	*NcoI*	*SpeI*
HindIII			
NcoI			
SpeI